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## **Performance of a cartridge based assay for the detection of clinically significant HPV infection – lessons from VALGENT (Validation of HPV Genotyping Tests)**

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### **Abstract**

The Validation of Genotyping Tests-HPV (VALGENT) studies offer an opportunity to clinically validate HPV assays for use in primary screening for cervical cancer and also provide a framework for the comparison of analytical and type-specific performance. Through VALGENT, we assessed the performance of the cartridge-based Xpert HPV Assay which detects 14 high-risk (HR) types and resolves HPV 16 and HPV 18/45.

Samples from women attending the UK cervical screening programme enriched with cytologically abnormal samples were collated. All had been previously tested by a clinically validated standard comparator test (SCT) – the GP5+6 EIA. Clinical sensitivity and specificity of the Xpert HPV for the detection of CIN2+ and CIN3+ relative to the SCT were assessed as was inter and intra lab reproducibility according to international criteria for test validation

(1). Type concordance for HPV 16 and HPV 18/45 between the Xpert and the SCT was also analysed.

Xpert HPV detected 94% of CIN2+ and 98% of CIN3+ lesions among all screened women and 90% of CIN2+ and 96% of CIN3+ in women of 30 years and older. The specificity for  $\leq$ CIN1 was 83% (95% CI 80-85%) in all women and 88% (95% CI 86-91%) in women 30 years and older. Inter and intra laboratory agreement for the Xpert was 98% and 97% respectively. The kappa agreement for HPV16 and HPV 18/45 between the CVRT (GP5+/6+ LMNX) and the Xpert was 0.92 and 0.91 respectively.

The clinical performance and reproducibility of Xpert is comparable to well established HPV assays and fulfils the criteria for use in primary cervical cancer screening.

## **Introduction**

Molecular HPV testing is being used increasingly for cervical cancer screening and management of (cytology) screen-positive women given the sensitivity and objectivity of this approach (2-4). As a consequence, the community is faced with an expanding portfolio of HPV tests which vary with respect to target, type-range, chemistry and level of automation, many of which are not associated with published, peer-reviewed evidence of performance (5). If HPV tests are used for the secondary prevention of cervical cancer, it is essential that they are clinically validated and this is particularly relevant given that HPV infection often clears without any associated morbidity.

International criteria have been established to evaluate the appropriateness of a new hrHPV DNA assay based on non-inferior sensitivity and specificity, compared to a clinically validated comparator assay, and high reproducibility (1). While they are not entirely perfect, they at least represent a consistent standard/benchmark via which performance can be assessed. One issue with validating an assay according to these criteria is that capturing representative samples which allow verification of non-inferior accuracy can be logistically challenging.

The VALGENT framework is an international collaboration designed to facilitate the clinical validation and comparison of HPV assays that offer genotyping capability (6). One of VALGENT's objectives is to allow the assessment of HPV assays according to the aforementioned clinical accuracy criteria, through the use of continuous samples from women participating in screening enriched with samples associated with cytopathological abnormalities.

The Xpert HPV Assay (Cepheid, Sunnyvale, CA, USA) is a PCR amplification assay which detects 14 HR-HPV types, offers limited genotyping (of HPV 16 and 18/45 as a duplex) and can provide a result in around one hour from sample addition. It differs from many competitor HPV assays in that the extraction and amplification processes are contained within an individual cartridge with minimal operator input other than addition of 1 ml of (un-manipulated) original sample (7). Initial reports on performance have been favourable when compared to FDA approved assays in both primary screening contexts and colposcopy settings (7-8). However, further data on performance pertaining to the Meijer criteria are outstanding, as are data on the performance/concordance of the type-specific aspects of the assay. The purpose of the present analysis was to address these gaps.

## **Materials and Methods**

### *Sample Collection*

Samples used for the present analysis constitute the VALGENT-2 panel. A detailed description of this panel has been described previously (9-10). In brief, archived samples were collated at the cytopathology laboratory at the Royal Infirmary of Edinburgh in Scotland which is one of the eight NHS laboratories that serves the Scottish Cervical Screening programme and processes around 70,000 samples per year. All samples were collected in PreservCyt™ liquid (Hologic, Bedford, MA, USA) from August 2012 to October 2012. The panel contained 1000 consecutive samples from the routinely screened population (the Scottish screening set) and 300 cytologically abnormal samples (the Scottish enrichment set). With respect to age,

as Scotland initiates screening at age 20, 419 samples were from women <30 years old, and 881 were from women aged 30 or more.

#### *Ethical approval*

Favourable ethical opinion for the project was provided by the West of Scotland Research Ethics Committee 4 - reference: 11/WS/0038

#### *Annotation of samples – HPV status*

All samples had been tested with GP5+/6+ PCR-EIA which was used as the standard comparator assay for clinical performance measurement, as per the Meijer criteria (1) and which used 0.5 ml of sample input. The amplicon generated via the GP5+/6+ PCR-EIA was also subjected to genotyping by the LMNX Genotyping kit HPV GP HR (GP5+/6+ LMNX;LBP LaboBiomedicalProducts, Rijswijk, the Netherlands) which uses Luminex xMAP technology for genotyping of the follow HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 26, 53, 73 and 82. The performance of the GP5+/6+ EIA and the genotyping provided by the GP5+/6+ LMNX on the VALGENT-2 panel is described in detail previously by Geraets et al 2014 (10). Both assays were performed at DDL Diagnostic Laboratory, Rijswijk, the Netherlands. The GP5+/6+ EIA and GP5+/6+ LMNX assays were performed between the April - May 2013 and the April - September 2013 respectively.

The Xpert HPV Assay (Xpert HPV, hereafter) testing was performed at the Scottish HPV Reference Laboratory in Edinburgh from the 16<sup>th</sup> April 2014 to the 14<sup>th</sup> of August 2014 according to manufacturer's instructions. Briefly, the assay is CE marked and detects 14 high-risk (HR) HPV types: (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) which are detected simultaneously via amplification of the E6 and E7 genes in five fluorescent channels: HPV16; HPV18/45; HPV31/33/35/52/58; HPV51/59; and HPV39/56/66/68a The assay also incorporates an human control gene: : hydroxymethylbilane synthase [HMBS]) as a sample and amplification validity check. A total of 1 ml of sample(s) was added to the cartridge before placement on to the Cepheid GeneXpert System.

#### *Annotation of samples - underlying pathology*

Cytology findings were reported according to the British Society for Clinical Cytopathology (BSCC) reporting guidelines with CIN nomenclature used to classify histological outcomes (11-13). Women with abnormal cytology results were managed according to guidelines defined by the UK NHS Cervical Screening Programme modified for use in the Scotland (13). Colposcopically directed biopsies were taken as routinely indicated. Clinical management was not influenced by HPV status.

#### *Age specific prevalence of HR-HPV according to Xpert HPV*

Prevalence and 95% confidence intervals of HR-HPV as measured by Xpert HPV was assessed in 5 year age-bands within the Scottish screening set.

#### *Assessment of clinical performance and comparison with a clinically validated standard comparator test (SCT)*

High grade disease was classed as histologically confirmed CIN2+ within 18 months of sample collection within the screening and enrichment set combined (n=101). No or low grade disease was assumed when a women either had 2 consecutive, cytologically negative samples across two screening rounds (average of 3 years and 11 months) or had  $\leq$ CIN1 after having a positive cytology screen (n=842). Sensitivity and specificity of Xpert HPV at the level of CIN2+ or CIN3+ was assessed for the overall sample set and also for women aged 30 and over separately. CIN3+ incorporated any cancer and also high grade glandular intraepithelial neoplasia. Relative sensitivity and specificity of Xpert HPV was compared to the SCT to determine whether these measures were lower than 0.90 and 0.98, respectively. Non inferiority was assessed by a one-sided statistical test for matched data (14). The null hypothesis of inferiority of the Xpert was rejected if  $P_{\text{non inf}} < 0.05$ . The  $\chi^2$  test of McNemar was used to assess differences between matched proportions and a  $p_{\text{McN}} > 0.05$  indicated that the sensitivity (or specificity) of Xpert HPV was not significantly different from GP5+/6+ EIA.

#### *Aggregation of VALGENT-2 data with existing UK Clinical data set*

The sample size for VALGENT2 was computed to assess the performance of assays in the Scottish Screening context where screening initiates aged 20 years. To bolster data on the performance of Xpert HPV in women aged 30 years and older, VALGENT data were combined with another UK screening based data set described in Cuzick et al 2015 (7). As the Meijer 2009 criteria are based on women >30, this aggregation (using two UK data sets) ensured the requisite number of women/outcomes to align with said criteria. The combined data sets is referred to as the “UK aggregated” data set. In Cuzick et al, 3408 samples obtained from the UK Cervical Screening Programme were tested with Xpert HPV, the COBAS HPV Test (Roche Molecular Systems, Pleasanton, CA, USA), and the Hybrid Capture 2 HPV Test (hc2) assay (Qiagen Ltd, Manchester, UK) which are both clinically validated HPV screening tests.

#### *Inter and Intra-lab reproducibility and type-specific agreement*

Inter and intra lab reproducibility was performed according to Meijer criteria which specifies that a minimum of 500 samples are assessed of which 30% are positive. Intra laboratory testing took place at AML laboratory (Antwerp, Belgium) and inter-laboratory testing between AML and the laboratory of the University Hospital of Ghent (Ghent, Belgium). A total of 510 samples collated at AML laboratories which had previously been tested with an in-house multiplex real-time PCR (15, 16) were assessed. For validation criteria to be satisfied, the 95% lower confidence bound of both agreements should be >87%, with a kappa value equal to or exceeding 0.5 (1).

Agreement between Xpert HPV and the GP5+/6+ LMNX for the detection of HPV 16 and HPV18/45 was evaluated using Cohens Kappa statistic. HPV 18/45 were reported as a combination by the Xpert HPV “agreement” of the GP5+/6+ LMNX was satisfied if the latter assay was positive for HPV 18 and/or 45

Supplementary data 1 provides an overview of the discrete sample set(s) used for the analyses described above.

## Results

### *Demographic, clinical and technical and characteristics of the VALGENT-2 panel*

When considering the Scottish screening and enriched population together – age ranged from 15 to 65 years with 881 being over 30 years of age. The average age (and range) in the screening and the enrichment set were 38 years (range, 18 to 68 years) and 31 years (range 19 to 62 years), respectively.

In the screening set, 10.2% of samples were cytologically abnormal and 9.2% and 1% had low-grade and high grade abnormalities respectively. In the enrichment set, samples were proactively selected for abnormality and incorporated 100 samples with borderline nuclear change, 100 with low grade dyskaryosis, and 100 with high grade dyskaryosis (moderate) or worse. Four samples were considered invalid with the Xpert HPV by generating a double negative (HPV and housekeeping control) result. Of these, 3 of 4 were from the screening set and 1 of 4 from the enrichment set. These samples were excluded from prevalence and accuracy assessments. Of the 1296 evaluable samples, outcomes were available for 943 - 101 were associated with histologically confirmed CIN2+ (55 of which were CIN3+) whereas 842 were associated with no disease (ie two consecutive negative cytology results or biopsy proven  $\leq$ CIN1).

### *HR-HPV prevalence as measured by the Xpert HPV in women attending for routine cervical screening in Scotland aged 20-60*

Overall HR-HPV prevalence by Xpert HPV was 18.0% and 11.1 % in women over 30 years and a total of 34 HPV 16 and 29 HPV 18/45 infections (3.4% and 2.9% respectively) were detected in the screening population. Prevalence of hrHPV infection by 5-year age group in the screening population is presented in Figure 1.

### *Clinical performance of Xpert for the detection of CIN2+*

Agreement between Xpert HPV and the GP5+/6+ EIA, stratified by disease outcomes (CIN2+, CIN3+ and  $\leq$ CIN1) for the VALGENT-2 data set is presented in Table 1 for of all ages and for women 30 years or older. Xpert HPV detected 94% (95% CI 86-98%) of CIN2+ and 98% (95% CI 90-100%) of CIN3+ lesions among all screened women and 93% (95% CI 80-98%) of CIN2+ and 96% (95% CI 79-100%) of CIN3+ in women of 30



years and older. The specificity for identifying women with  $\leq$ CIN1 was 83% (95% CI 80-85%) and 88% (95% CI 86-91%), in all women and in women 30 and older, respectively. Table 2 details the absolute and relative accuracy of the Xpert compared to the SCT for both the VALGENT data set and the aggregated dataset. In addition, cross tabulations of the Xpert vs the SCT are provided in Table 3 for the combined data set. The sensitivity and specificity of Xpert HPV was not significantly different from GP5+/6+ EIA (Table 2; 95% CI around the relative accuracy measures always included unity and  $p_{McN}$  was never significant). Non-inferior sensitivity and specificity of Xpert HPV compared to GP5+/6+ EIA was demonstrated for all outcomes, except for women of 30 and older, where it was inferior with respect to sensitivity for CIN2+ and CIN3+. However, by combining, VALGENT-2 data with those of Cuzick et al 2015 (7), the hypothesis of inferiority was rejected for women aged  $\geq 30$ .

#### *Type specific agreement between Xpert HPV and the GP5+/6+ LMNX Assay*

In the screening and enrichment sets combined, 118 HPV 16 infections were detected by Xpert HPV compared to 110 by the GP5+/6+ LMNX. A total of 106 samples were HPV 16 positive for both tests whereas, 12 were HPV 16 positive for Xpert HPV only and 4 for GP5+/6+ LMNX only. The overall concordance for HPV16 was 98.8% (98.0-99.3%) with a kappa value was 0.923 (95% CI 0.886, 0.960). Concordance for presence of HPV18/45 was 99.2% (95% CI 98.5-99.6%) with a kappa of 0.915 (95% CI 0.865-0.965).

#### *Inter-laboratory and Intra-laboratory agreement for hrHPV testing with Xpert HPV*

**A total of 510 samples were assessed for intra and inter laboratory reproducibility.**

The overall intra-laboratory concordance of hrHPV positivity was 96.9% (95% CI 95.0-98.2%) with a kappa of 0.925 (95% CI 0.888-0.961) whereas the inter-laboratory concordance between initial testing in Antwerp and retesting in Ghent was 97.8% (95% CI 96.2-98.9%) with a kappa of 0.948 (95% CI 0.917-0.978).

## Discussion

The Xpert HPV is a cartridge based test which detects 14 HR-HPV types and offers concurrent limited typing capability. It is a rapid technically undemanding assay and integrates extraction and detection within an individual cartridge. Different levels of instrument throughput are available for the assay; from single-module systems to 80-module systems enabling applications within point of care to settings to high-throughput centralised service laboratories.

As a relatively new assay there is, understandably, less data on clinical performance compared to more established tests. This said, the data available thus far have been encouraging; Einstein et al (2014), assessed the clinical performance of Xpert HPV in 697 samples obtained from colposcopy referral populations across 7 US sites, with performance compared to the COBAS HPV test and the hc2. The Xpert HPV showed comparable sensitivity for CIN2+ compared with the COBAS HPV test and hc2 respectively whereas the highest specificity was conferred by the hc2 followed by Xpert HPV and then the COBAS HPV test – leading the authors to conclude that the Xpert HPV performance was “comparable to that of currently available clinically validated tests” (8).

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A further analysis of this colposcopy study was performed by Castle et al (2015). Here, the authors assessed assay agreement across two samples, and demonstrated a high agreement of 95% y. % (17). This observation of high agreement reconciles with the present study where inter and intra-laboratory agreement was also high.

To our knowledge, this is the first study to assess the performance of Xpert HPV using the Meijer criteria and accordingly, Xpert HPV fulfils the criteria with respect to sensitivity and specificity relative to a clinically validated standard comparator test and also inter and intra-laboratory reproducibility. This assessment builds on the previous work of Cuzick et al (2015) where a total of 3408 prospective samples derived from women attending for routine cervical screening within the UK programme and collated across 3 sites were tested with Xpert HPV, hc2 and COBAS HPV test. Respective sensitivities of the assays for CIN2 + were 98.7%, 97.5% and

98.7% with specificities of 82.3%, 82.7% and 82.3% (7). Indeed aggregation of this data with the VALGENT-2 series allowed more precise assessment of the performance of Xpert HPV in women over 30 – an important consideration given that many HPV based primary screening protocols stipulate 30 as a minimum age for application. Consistent, with previous work (9) - the data also demonstrate, the high prevalence of HR-HPV in the UK, particularly in young women, emphasising the need for appropriate triage strategies in an era of HPV primary screening (18).

Like many other HPV assays, Xpert HPV offers limited typing capability – HPV 16 and HPV 18/45. Type specific agreement for HPV 16 and HPV 18/45 between the Xpert and the GP5+/6+ LMNX was high although as the Xpert HPV does not delineate between 18/45 separately the concordance between it and the LMNX which does provide individual resolution is somewhat artificial. Further outputs from the VALGENT studies will generate more inter-test comparisons for all assays used within the projects so that type specific discordances and their relevance can be examined more comprehensively.

There are caveats to the analysis – as the timeframe of testing for the GP5+/6+ EIA, LMNX and Xpert HPV were not exactly matched it is feasible that this may have influenced the results. Furthermore, storage of VALGENT-2 samples prior to testing may affect assay performance to an extent. However, if storage were to have a deleterious impact on assay-detection a lower sensitivity may be anticipated whereas the sensitivity of Xpert HPV for CIN2+ and CIN3+ was high and equivalent or higher to that described in the prospective series (7,8). In addition as biopsies were only indicated as a consequence of preceding abnormal cytology, some CIN2+ may have been missed – although further longitudinal follow up of the cohort – which is planned will address this at least in part.

To conclude, the clinical performance and reproducibility of Xpert HPV is comparable to standard comparator tests (the hc2, and the GP5+/6+ EIA) which demonstrated better protection against cervical cancer than cytology alone (1). Therefore, Xpert

HPV may be added to the list of HPV assays considered validated for primary screening for cervical cancer (19)

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**Competing interests:** Although industrial funding was used for this evaluation, it was investigator-led and the analysis performed independently. KC has received project funding and/or consumables to carry out assay evaluations from HOLOGIC, Qiagen, Roche, NorChip, Cepheid, GeneFirst, GSK & Abbott in the past. . JC is on advisory boards/speakers bureaux for [BD](#), [Abbott](#), [Hologic](#), [Cepheid](#), [Genera](#), [Merck](#). The Scientific Institute of Public Health, where MA is employed, received support from Cepheid for methodological and statistical work as foreseen in the VALGENT Network (6). EP provides [sporadic scientific advice for Roche and Fujirebio](#). No other authors declare a conflict of interest.

**Contributions:** KC was local (NHS Lothian) principal investigator for the study and created the manuscript drafts, DG and WQ were responsible for delivery and analysis of the sample panel with the standard comparator test. JC and LC contributed data and analytical support with respect to the project outlined in Cuzick et al (7). CM organised and delivered laboratory testing using the Xpert assay. DVB and EP performed the intra and inter laboratory testing elements. MA is chief investigator of the VALGENT projects and performed statistical analysis. All authors provided critical comment to manuscript drafts.

|          |                | Xpert |     |       |  |            |                | Xpert |     |       |
|----------|----------------|-------|-----|-------|--|------------|----------------|-------|-----|-------|
|          | GP5+/6+<br>EIA | +     | -   | total |  |            | GP5+/6+<br>EIA | +     | -   | total |
| All ages |                |       |     |       |  | >=30 years |                |       |     |       |
| CIN2+    | +              | 93    | 2   | 95    |  | CIN2+      | +              | 36    | 1   | 37    |
|          | -              | 2     | 4   | 6     |  |            | -              | 2     | 2   | 4     |
|          | Total          | 95    | 6   | 101   |  |            | Total          | 38    | 3   | 41    |
|          |                |       |     |       |  |            |                |       |     |       |
| CIN3+    | +              | 54    | 0   | 54    |  | CIN3+      |                | 23    | 0   | 23    |
|          | -              | 0     | 1   | 1     |  |            | -              | 0     | 1   | 1     |
|          | Total          | 54    | 1   | 55    |  |            | Total          | 23    | 1   | 24    |
|          |                |       |     |       |  |            |                |       |     |       |
| ≤CIN1    | +              | 116   | 25  | 141   |  | ≤CIN1      | +              | 55    | 14  | 69    |
|          | -              | 30    | 671 | 701   |  |            | -              | 21    | 562 | 583   |
|          | Total          | 146   | 696 | 842   |  |            | Total          | 76    | 576 | 652   |

**Table 1:** HR-HPV positivity of Xpert vs GP5+6+ EIA in women attending for routine screening in Scotland (ie VALGENT 2 data set) for whom outcome data were available. Cross-tabulations are stratified according to women having CIN2+, CIN3+ or two consecutive negative cytology results or ≤CIN1. The left hand section contains women of all ages (n=943) whereas the right hand section contains women ≥30 years of age (n=693).

| Age group      | Measure     | Outcome (N)  | Absolute accuracy  |                    | Relative accuracy     | p McN | p n.inf |
|----------------|-------------|--------------|--------------------|--------------------|-----------------------|-------|---------|
|                |             |              | Xpert              | SCT                | Xpert vs SCT , 95% CI |       |         |
| VALGENT 2      |             |              |                    |                    |                       |       |         |
| All ages       | Sensitivity | CIN2+ (101)  | 94.1% (87.5-97.8%) | 94.1% (87.5-97.8%) | 1.000 (0.960, 1.042)  | 1.00  | 0.0017  |
|                | Sensitivity | CIN3+ (55)   | 98.2% (90.3-100%)  | 98.2% (90.3-100%)  | 1.000 (. - .)         | 1.00  | 0.0072  |
|                | Specificity | ≤CIN1 (842)  | 82.7% (79.9-85.1%) | 83.2% (80.6-85.7%) | 0.993 (0.972-1.014)   | 0.50  | <0.0001 |
| ≥30 years      | Sensitivity | CIN2+ (41)   | 90.2% (76.9-97.3%) | 92.7% (80.1-98.5%) | 0.974 (0.889-1.066)   | 1.00  | 0.0951  |
|                | Sensitivity | CIN3+ (24)   | 95.8% (78.9-99.9%) | 95.8% (78.9-99.9%) | 1.000 (. - .)         | 1.00  | 0.055   |
|                | Specificity | ≤CIN1 (652)  | 88.3% (85.6-90.7%) | 89.4% (86.8-91.7%) | 0.988 (0.968-1.008)   | 0.24  | <0.0001 |
| AGGREGATED SET |             |              |                    |                    |                       |       |         |
| All ages       | Sensitivity | CIN2+ (180)  | 96.1% (92.2-98.4%) | 96.1% (92.2-98.4%) | 1.000 (0.978-1.023)   | 1.00  | <0.0001 |
|                | Sensitivity | CIN3+ (102)  | 99.0% (94.7-100%)  | 99.0% (94.7-100%)  | 1.000 (. - .)         | 1.00  | 0.0004  |
|                | Specificity | ≤CIN1 (4171) | 82.5% (81.3-83.6%) | 82.1% (80.9-83.3%) | 1.001 (0.992-1.011)   | 0.76  | <0.0001 |
| ≥30 years      | Sensitivity | CIN2+ (68)   | 92.6% (83.7-97.6%) | 94.1% (85.6-98.4%) | 0.984 (0.931-1.040)   | 1.00  | 0.019   |
|                | Sensitivity | CIN3+ (38)   | 97.4% (86.2-99.9%) | 97.4% (86.2-99.9%) | 1.000 (. - .)         | 1.00  | 0.021   |
|                | Specificity | ≤CIN1 (3206) | 88.1% (86.9-89.2%) | 87.5% (86.3-88.6%) | 1.006 (0.997-1.016)   | 0.18  | <0.0001 |

**Table 2 . Clinical performance of the Xpert compared to a standard, clinically validated comparator tests (hc2 or GP5+6+ EIA)** Absolute sensitivity of the tests for CIN2+ or CIN3+ and specificity for ≤ CIN1 are presented as is relative accuracy of Xpert HPV compared to the comparator tests. The statistical tests in the last two columns verify differences (p McNemar) or non-inferiority (P n.inf). The upper six rows show accuracy measures according to the VALGENT 2 set where the comparator test was GP5+/6+ EIA,. The aggregated data set incorporates VALGENT 2 and data from Cuzick et al (7) where the comparator test was hc2

|                                 | Xpert |      |       |
|---------------------------------|-------|------|-------|
| HC2 or<br>GP5+/6+ EIA           | +     | -    | Total |
| <b><i>CIN2+ Age ≥30</i></b>     |       |      |       |
| +                               | 62    | 2    | 64    |
| -                               | 1     | 3    | 4     |
| <b>Total</b>                    | 63    | 5    | 68    |
| <b><i>&lt;CIN2 Age ≥30</i></b>  |       |      |       |
| +                               | 300   | 100  | 400   |
| -                               | 82    | 2724 | 2806  |
| <b>Total</b>                    | 382   | 2824 | 3206  |
| <b><i>CIN2+ All Ages</i></b>    |       |      |       |
| +                               | 171   | 2    | 173   |
| -                               | 2     | 5    | 7     |
| <b>Total</b>                    | 173   | 7    | 180   |
| <b><i>&lt;CIN2 All ages</i></b> |       |      |       |
| +                               | 611   | 130  | 741   |
| -                               | 125   | 3305 | 3430  |
| <b>Total</b>                    | 736   | 3435 | 4171  |

**Table 3.** HR-HPV positivity of Xpert vs clinically validated reference test(s) using aggregated data from VALGENT 2 and Cuzick et al (2015) <sup>7</sup> Data are stratified according to presence or absence of underlying disease and separately for women of all ages and women ≥30

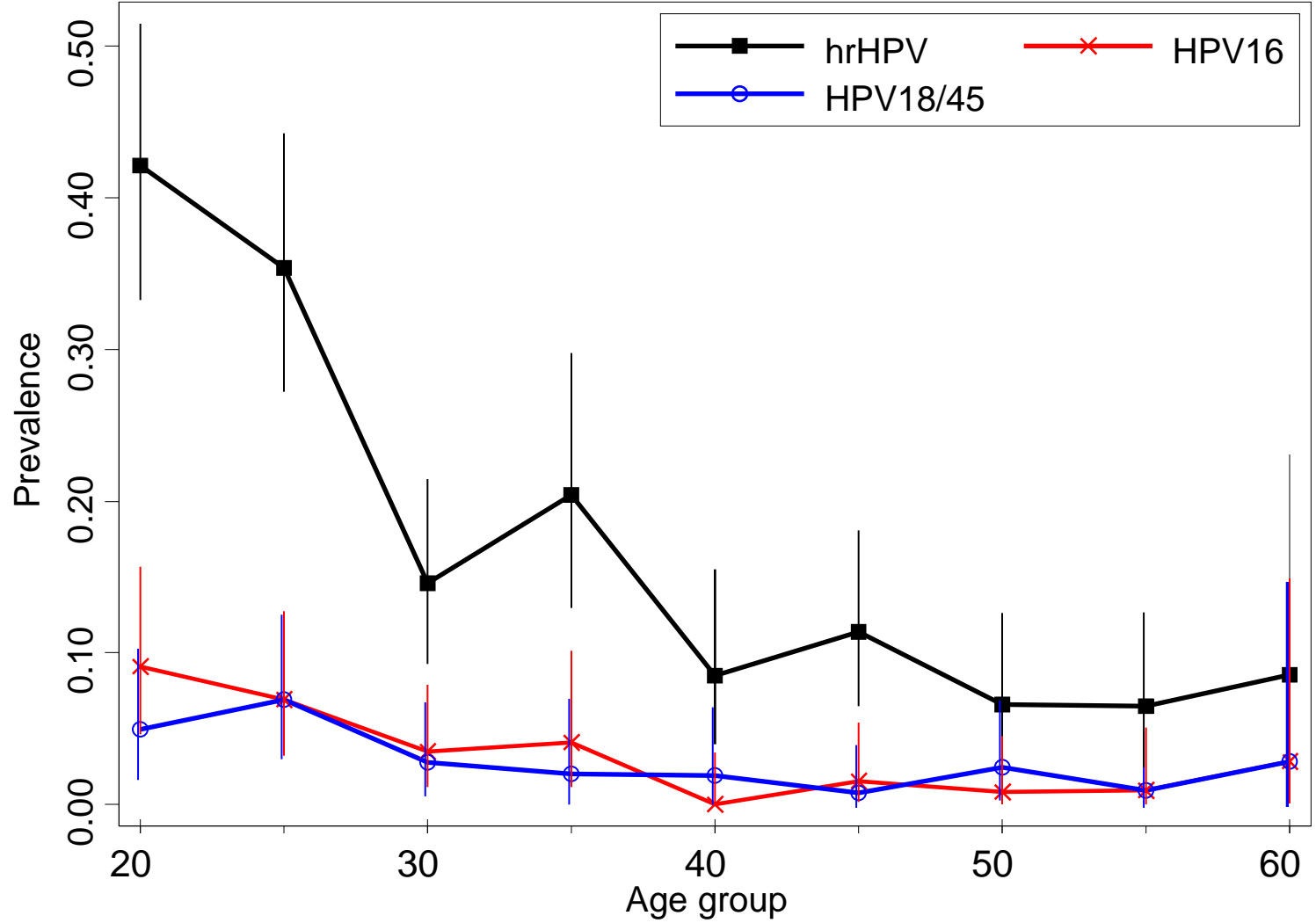
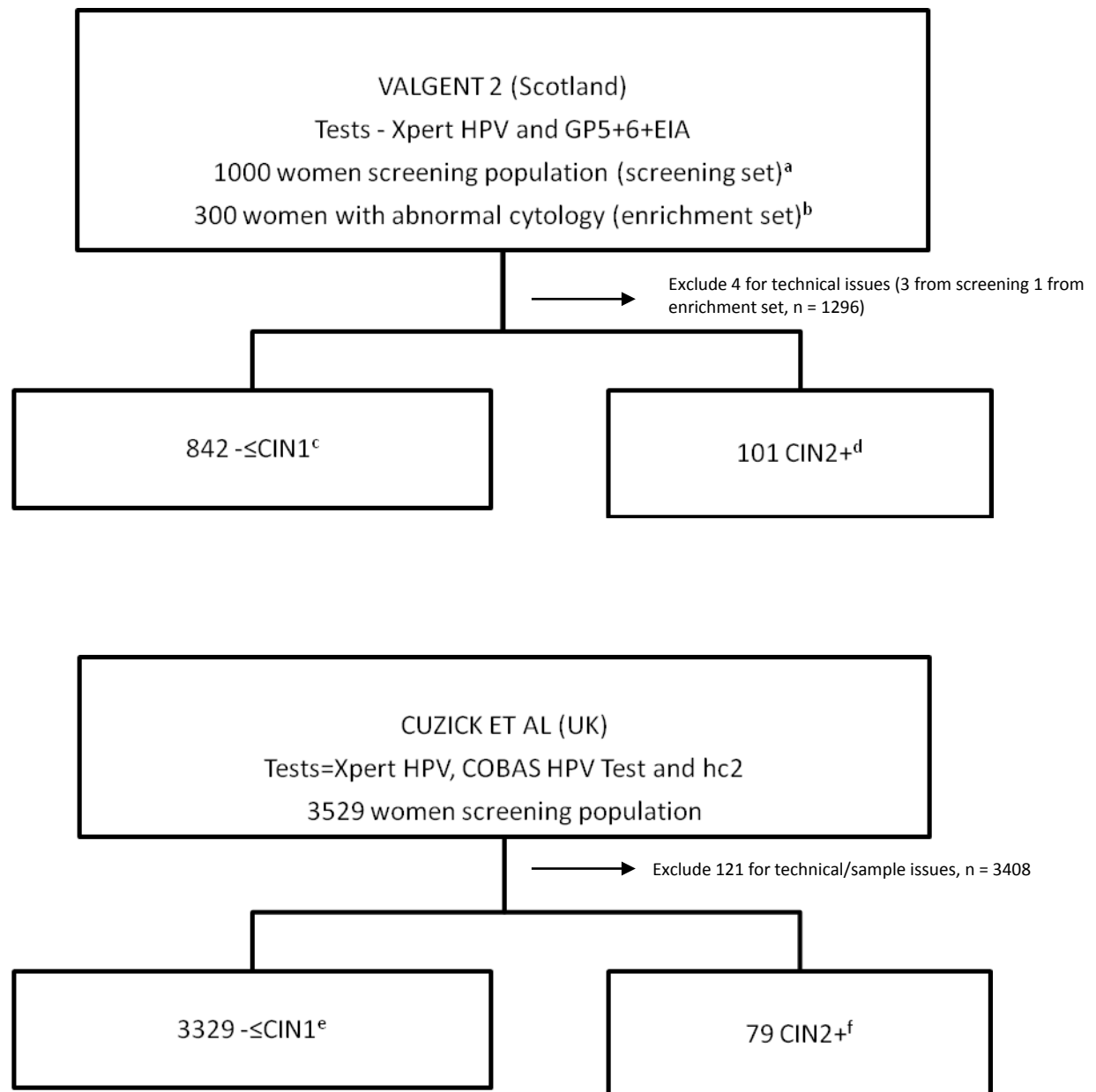


Figure 1: HR-HPV prevalence using the Xpert HPV in the routinely screened population in Scotland (aged 20-60)





**Supplementary data 1. Overview of samples used for the assessment of prevalence, type specific concordance and clinical performance of the Xpert HPV.**

Of the VALGENT 2 set (upper image) 1296 samples were technically valid. HR-HPV prevalence (as detailed in figure 1) was assessed using “a” and type specific concordance for HPV 16 and 18/45 was assessed using a & b. A total of 943 had follow up/outcome data and (c & d) were used for the assessment of clinical performance of the Xpert HPV in absolute terms and also in relation to a clinically validated reference test. Clinical performance of the Xpert was also assessed by aggregating data from VALGENT 2 with that of Cuzick et al <sup>7</sup> (lower image) where a total of 3408 women had follow up/outcome data related to the Xpert HPV and a clinically validated reference test, permitting assessment of 4351 outcomes (c-f). Note the samples used for inter and intra-laboratory agreement were outside of the above sample pool with this element of the analysis performed between Ghent and Antwerp in Belgium.

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